PCRarray 2.0
The second Generation of PCR Array

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qLight™ qPCR Gene Expression Assay System

Simple, Fast & Reliable

(Version 1.2)

For Research Use Only
Not For Use in Diagnostics

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2. Materials Provided

Validated primer mix in array plate or in tubes

1). Pre-designed array qPCR kit: 96-well qPCR array plates with sets of PCR primers for up to 84 target genes you have ordered;

Shipping condition: Ambient temperature

Storage condition: 4 ^{o}C for 6 months

2). Custom array qPCR Kit: 96-well qPCR array plates with sets of PCR primers per your request;

Shipping condition: Ambient temperature

Storage condition: 4 ^{o}C for 6 months

3). Single gene qPCR kit: Primer mix solutions in 1.5ml microtube(s) for the gene of your interest. One tube contains 400 µl primer mixes, enough for 200 reactions

Shipping condition: cold with blue ice

Storage condition: 4 ^{o}C for 6 months

4). Reference & QC Array Plate Kit (optional): as an option for quality control with internal reference reactions and positive QC control reactions array; to be used with:

a. For custom array kits with target gene number less than 49

b. For single gene assay primer mix kit

Shipping condition: Ambient temperature

Storage condition: 4 ^{o}C for 6 months

3. Additional Materials Required

Note: The following reagents are **mandatory** for optimal results. The counterparts or equivalent kits from other vendors are not recommended due to unique features of our system design.

1). qLight™ Genomic DNA-free First-Strand cDNA Synthesis Kit: Cat# RT03

2). qLight™ SYBR green qPCR Master Mix: refer to the table below for the Master-mix products for your PCR cycler. Master-mix is designed based on the reference dye per manufacturers of common PCR cyclers.

Master Mix Type	Cat No	Size	Real-Time PCR Cyclers
qLight™ /ROX SYBR green Master Mix (MR1)	MR1-02	2X 96-well plates/ 200 rxn (2X1.3mL)	All ABI & Stratagene Instrumentation, Eppendorf Mastercycler® ep realplex Instruments with ROX filter set
	MR1-12	12X 96-well plates/ 200 rxn (12X1.3mL)	
	MR1-24	24X 96-well plates/ 200 rxn (24X1.3mL)	
qLight™ / Fluorescein SYBR Green Master Mix (MF2)	MF2-02	2X 96-well plates/ 200 rxn (2X1.3mL)	BioRad iCylcer®, MyiQ®, and iQ5 Instrumentation
	MF2-12	12X 96-well plates/ 200 rxn (12X1.3mL)	
	MF2-24	24X 96-well plates/ 200 rxn (24X1.3mL)	
qLight™ SYBR Green Master Mix (MM3 No reference dye)	MN3-02	2X 96-well plates/ 200 rxn (2X1.3mL)	BioRad Opticon, Opticon 2 & Chromo 4, Roche LightCycler® 480 System, Eppendorf Mastercycler® ep realplex Instruments without ROX filter set
	MN3-12	12X 96-well plates/ 200 rxn (12X1.3mL)	
	MN3-24	24X 96-well plates/ 200 rxn (24X1.3mL)	

4. Introduction

qLight™ gene expression qPCR assay system is a simple, fast and reliable real-time PCR system. It has three major product components with compatible design to assuare quality and performance with convenience. These components are reliable templates using our genomic DNA (gDNA)-free first-strand cDNA synthesis system, performance-validated primers designed with TG primer design software with genome-wide design capacity, and qLight™ SYBR green real-time PCR Master-mix. Following products are offered with qLight™ gene expression qPCR assay system:

1). Catalogued qLight™ pathway qPCR array gene expression profiling kit

- Sets of designed primers for pre-selected pathway genes (up to 84 genes) dried down to 96-well plate
- With internal reference reactions and external positive reactions monitoring RT reaction and PCR reaction
- Simply add the master-mix and cDNA cocktail for the PCR reactions.

2). Custom qPCR array gene expression profiling kit

- The flexibility to design your array with benefits of our manufacturing consistency and internal reference and QC standard
- Simply add the master-mix and cDNA cocktail to your custom qPCR array plates (see page for array layout and the protocol)

3). Single gene expression qPCR assay kit

- Convenience to perform single gene expression assays (see page for the protocol)

4.) Reference and QC Array:

- Internal reference reactions and external control reactions
- To be used with customer qPCR array products and single gene assays
- Eight samples can be analyzed simultaneously (see page and page)

Benefit

- Simple genomic DNA-free first-strand cDNA synthesis to eliminate contamination concern of genomic DNA
- High PCR performance with validated primers and matched PCR Master-mix for your cycler
- Built-in controls to monitor quality and performance
- Simple & Fast procedure to profile up to 84 gene expression in 2.5 hours
- Data analysis template offered for you to obtain your results within minutes.

The simple three-step procedure (See Fig 1) – total time 3 hours

Step 1: First-strand cDNA synthesis in 35 minutes from RNA to cDNA

Step 2: Setup PCR reaction in 10-15 minutes:

i). Prepare PCR cocktail by mixing synthesized cDNA with qLight™ qPCR master-mix;

ii). dispense the cocktail to the selected qLight™ array plate.

Step 3: Run real-time PCR reaction in about two hours

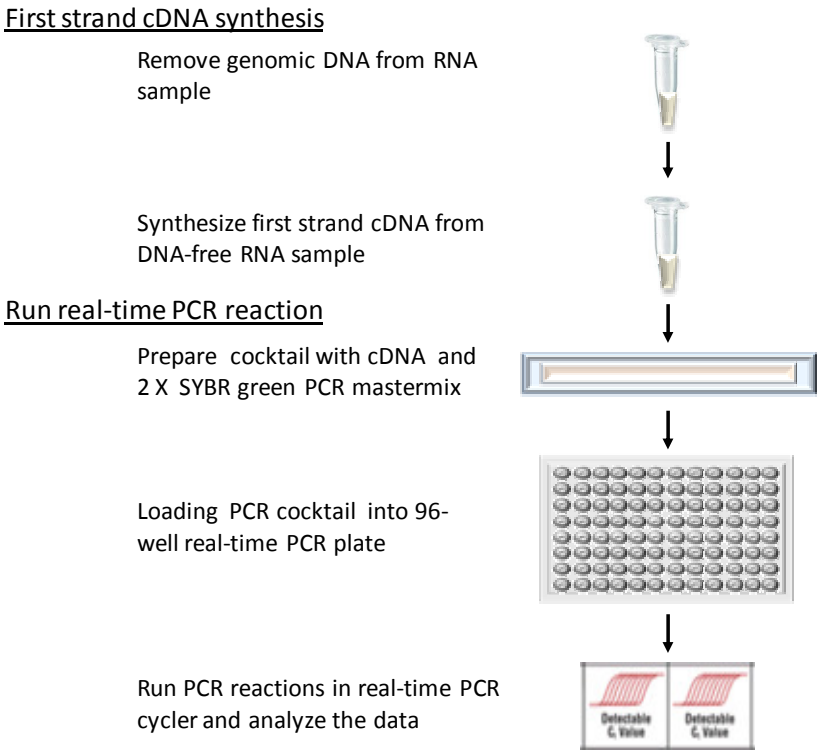
First strand cDNA synthesis
Remove genomic DNA from RNA sample
Synthesize first strand cDNA from DNA-free RNA sample
Run real-time PCR reaction
Prepare cocktail with cDNA and 2 X SYBR green PCR mastermix
Loading PCR cocktail into 96-well real-time PCR plate
Run PCR reactions in real-time PCR cycler and analyze the data
Detectable C. Value
Detectable C. Value

Fig 1. Array profiling procedure flowchart

Array plate layout and data analysis

A typical array plate contains following components (See Fig 2)

1. Wells A1 to G12: Reactions of 84 target genes to be analyzed in your samples;
2. Wells H1 to H8: Reactions of 4 housekeeping genes in duplicate as internal reference controls;
3. Wells H9 and H10: 5' and 3' portion reactions for same housekeeping gene to monitor if input RNA sample is integrate ;

4. Well 11: external reverse transcription positive control reaction (RTPC) to monitor RT reaction of input smaples;

5. Well 12: external PCR positive control reaction (PCRPC) to monitor the PCR reaction.

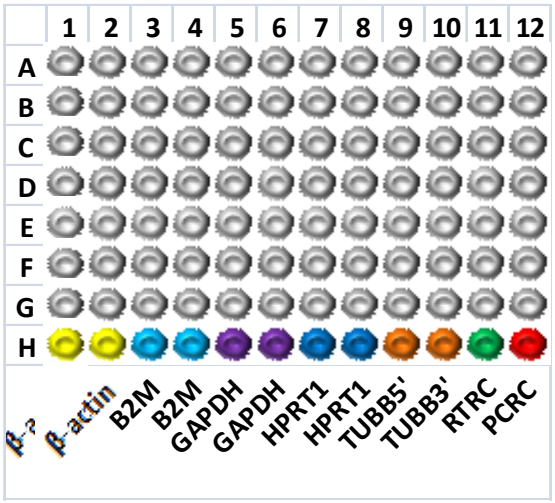

Fig 2. A typical gene expression signature array plate layout

Data analysis template is developed based on ΔΔCt method based on the fold changes in gene expression between your test samples and control samples. Following simple steps below, the relative expression level (fold change) is automatically computed between two samples for comparison

1. Download the analysis template from our website (link here):

2. Enter the raw Ct numbers from the PCR reaction plates into the corresponding rows and columns of the analysis template by simple copying and pasting, following the instruction described in the instruction sheet

3. The RT positive control reaction (RTPC) and PCR positive reaction control reaction (PCRPC) results are displayed in the "QC control sheet".

4. The target gene results will be automatically displayed in "result sheet". Two-fold changes are built-in default positive changes

5. Protocol

Note: The RT kit and the SYBR green master-mix are **mandatory** for desired performance. Other commercial equivalent products are not expected to work due to the unique designs of primer sets and the control layout.

1). cDNA synthesis using qLight™ Genomic DNA-free First-Strand cDNA Synthesis Kit (Cat# RT-01):

Note: The protocol and reagents are not compatible with any counterpart products from other vendors.

a. Pretreatment of RNA sample to remove contaminated genomic DNA

i. Add the following reagents to a PCR reaction tube for each RNA sample:

Components	**Volume**
RNA (0.1-2µg)	1 µl
10 X Buffer	1 µl
DNase	1 µl
DNase-, RNase-free ddH2O	7 µl
Total	**10 µl**

ii. Incubate in a thermal cycler at 42 ^{o}C for 5 minutes, then immediately put the tube on ice for at least 1 minute.

b. Reverse transcription reaction:

i. Prepare the cocktails following the table bellow for different sample numbers

	Reaction number			
Component	1 X	2 X	4 X	8 X
DNase-, Rnase-free ddH2O	3 µl	6 µl	12 µl	24 µl
0ligo mix	1 µl	2 µl	4 µl	8 µl
5X buffer	4 µl	8 µl	16 µl	32 µl
RT enzyme Mix	2 µl	4 µl	8 µl	16 µl
Total	**10 µl**	**20 µl**	**40 µl**	**80 µl**

ii. Mix well. Add 10 µl of the cocktail to the each pretreatment reaction for each sample

iii. Gently mix, incubate at 42 °C for 20 minutes, then followed by at 95 °C for 5 minutes

iv. Add 95 µl DNase-, RNase-free ddH2O to each reaction, mix well and place it on ice until use. Or store at -20 °C

2). Assemble 96-well PCR array plate reactions & run real-time PCR plate

i. To a clean reservoir, add:

Component	**Volume**
DNase-, Rnase-free ddH_2O	985 µL
Targetgreen SYBR green qPCR Master Mix	1100 µL
Diluted reverse transcription product	115 µL
Total	**2200 µL**

ii. Mix well, add 20 µl of the mix to each well of the array plate using a multi-channel pipette

iii. Seal the well with the provided transparent film or the 8-cap strip

iv. Spin briefly to collect the mixture solution to the well-bottom

v. Place the plate on PCR cycler and run the reaction using the cycling program below:

	Temperature	**Time**	**Cycling number**
Initial denaturation & Taq activation stage	95 °C	10 min	1
Cycling stage			40
Denaturation	96 °C	15 sec	
Annealing & extension	60 °C	1 min	
Dissociation curve (Melting curve) stage	Add the stage following cyclers' instruction		1

3). Data analysis:

a. Export raw Ct values from your PCR run file after the setup of the threshold and baseline as below:

   i. Set up threshold at 0.2

   ii. Set up baseline up the starting at cycle 3 and stop at cycle 15.

b. Download software template fitting to these array layout are available our website.

c. Follow the instruction of data analysis template for your array layout, enter the Ct values of the target gene array plate run, along with those of reference reaction and QC control array plate run.